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16. ABSTRACT

Cyclopenta-fused PAH are a novel class of environmental PAH of which the most well known example is cyclopenta(cd)pyrene. The fusion of an unsaturated cyclopenta-ring on a PAH in general, markedly enhances its activity as a gene mutagen in bacteria and cultured mammalian cells, a cell transforming agent in rodent cells and a mouse skin tumor initiator. A series of four cyclopenta-fused isomers of benz(a)anthracene and the cyclopenta-fused isomers of anthracene and phenanthrene were studied with respect to the major rat liver microsomal metabolites, their activity as gene mutagens in S. typhimurium and Chinese hamster V79 cells and their ability to morphologically transform C3H10T1/2CL8 mouse embryo fibroblasts. For all six isomers, the dihydrodiol arising from oxidation and hydration at the cyclopenta-ring was a major pathway in Aroclor-1254 induced rat liver microsomes. All six isomers were active in mutating S. typhimurium and the four benz(a)anthracene isomers active in mutating V79 cells at the HGPRT locus. In general they were all more active than their parent unsubstituted PAH and several were more active than benzo(a)pyrene. Three of the four benz(a)-anthracene isomers transformed C3H10T1/2 cells, the other three cyclopenta-PAH were inactive. This evidence suggests that oxidation at cyclopenta-ring is a major pathway in the biotransformation and bioactivation of cyclopenta-PAH.

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METABOLIC ACTIVATION PATHWAYS OF CYCLOPENTA-FUSED PAH AND THEIR RELATIONSHIP TO GENETIC AND CARCINOGENIC ACTIVITY

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INTRODUCTION

Cyclopenta-polycyclic aromatic hydrocarbons (CP-PAH) are a unique class of environmental non-alternant PAH. The fusion of an ethylene fragment to a PAH to form a new PAH with an unsaturated five-membered ring not only structurally alters the molecule but has dramatic effects on the electronic distribution, the ability of the PAH to be metabolized, and the genetic and carcinogenic activity. The relationships between parent PAH and CP-PAH are found in Table 1.

TABLE 1. Parent PAH and the related Cyclopenta-PAH

Parent	CP-PAH	Abbreviation
Anthracene	Aceanthrylene	AA
Phenanthrene	Acephenanthrylene	AP
Benz(a)anthracene	Benz(e)aceanthrylene	B(e)A
	Benz(j)aceanthrylene	B(j)A
	Benz(k)acephenanthrylene	B(k)A
	Benz(l)aceanthrylene	B(l)A
Chrysene	Benz(k)acephenanthrylene	
Tetracene	Benz(d)aceanthrylene	
	Benz(k)aceanthrylene	
Benzo(c)phenanthrene	Benz(e)acephenanthrylene	
Pyrene	Cyclopenta(cd)pyrene	CPP

To date our laboratory has studied the CP-PAH related to anthracene (A), phenanthrene (P), benz(a)anthracene (BA) and pyrene (PY). Current investigations are underway with the CP-PAH related to chrysene, tetracene, and benzo(c)-phenanthrene.

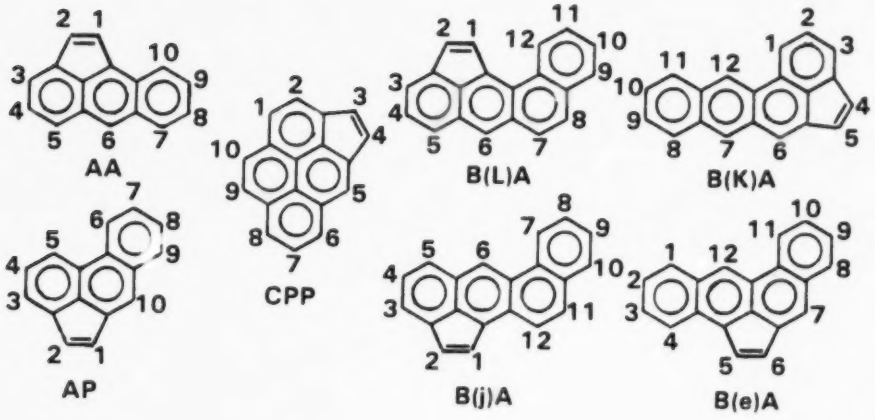

Figure 1. Structures of CP-PAH which have been examined for genotoxic activity

The most well studied CP-PAH is cyclopenta(cd)pyrene (CPP), a ubiquitous environmental contaminant. Recent studies have indicated that, like CPP, other CP-PAH are found in the environment (Table 2). B(e)A, B(j)A, CPP, and AP have been identified in coal emission extracts and B(l)A, AP, and CPP have been identified in wood smoke emissions.

A large volume of experimental data is available on the metabolic activities of PAH. For those PAH not substituted with alkyl groups, insertion of an oxygen into a carbon-carbon double bond to form an arene oxide is the first step in the metabolic activation. These arene

TABLE 2. Environmental Occurrence of Cyclopenta-PAH

CP-PAH	Sources	Reference
CPP	Automobile exhausts Coal combustion Wood combustion Pitch, carbon black Tobacco smoke Grilled foods	Karcher et al., 1985
B(e)A	Coal combustion	Y. Jacob[a]
B(j)A	Coal combustion	Y. Jacob[a]
B(l)A	Wood smoke	R. Kamens[a]
AP	Coal combustion	Y. Jacob[a]
	Wood smoke	R. Kamens[a]

[a]personal communication

oxides can rearrange to form phenols, be hydrated by microsomal epoxide hydrolase or can react with biological molecules including DNA. A number of PAH initially metabolized to dihydrodiols can be oxidized a second time to form diol-epoxides. The diol-epoxides of benzo(a)pyrene [B(a)P], BA, 3-methylcholanthrene (3MC), chrysene, and other PAH are highly genotoxic and react with DNA to form covalent PAH-DNA adducts. Further metabolism of phenols and dihydrodiols can occur with the formation of quinones and various conjugates of glucuronic acid, sulfate and sulfhydryl containing cellular constituents (IARC, 1983 Singer and Grunberger, 1983).

Using Aroclor-1254 induced rat liver S9 as a metabolic activation system, the genotoxic activities of a series of CP-PAH have been determined using histidine reversion in Salmonella typhimurium, strain TA 98, 6-thioguanine resistance in Chinese hamster V79 cells or trifluorothymidine resistance in mouse lymphoma L5178Y cells (Table 3). Also examined for several CP-PAH were their ability to induce morphological transformation in C3H10T1/2CL8 mouse embryo fibroblasts and skin papillomas in SENCAR mice promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA). In comparison to B(a)P or 3MC all the CP-PAH examined except for B(k)A were more active in inducing reversion in strain TA 98 of S. typhimurium. Characteristic of most CP-PAH is the lower requirement of S9 microsomal protein/plate for maximal reversion compared to B(a)P or 3MC suggesting a one-step

epoxidation as the activation pathway (Eisenstadt and Gold, 1978, Nesnow et al., 1984b). Using mammalian cell gene mutation in V79 cells, B(l)A, B(e)A, and B(j)A were all more active as gene mutagens than B(a)P. Similarly, as a mouse skin tumor initiator, B(l)A was four times as active as B(a)P while B(e)A was equivalent in activity. The increased activity of B(l)A compared to B(a)P was not evident in C3H10T1/2CL8 cells. Using morphological transformation as the genotoxic endpoint, B(e)A and B(l)A were 10-fold less active than B(a)P while B(j)A was equivalent in activity to B(a)P. B(k)A, AA, and AP were inactive.

TABLE 3. Summary of Genotoxic Activities of Cyclopenta-PAH and PAH

	Effect/Organism			
	Bacterial Cell Gene Mutation/ *S. typhimurium* TA98	Mammalian Cell Gene Mutation/ V79 or L5178Y Cells	Morphological Transformation/ C3H10T1/2CL8 or BALB/c Cells	Mouse Skin Tumor Initiation/ SENCAR or CD1 Mice
A	-[a]		-[a]	
AA	++[b]		-[c]	
P	-[a]		-[a]	
AP	+[b,d]		-[c]	
BA	+[a]	+[a]	-[e]	+[f]
B(j)A	++[g]	++[g]	++[c]	
B(e)A	++[g]	++[g]	+[c]	++[h]
B(l)A	++[g]	++[g]	+[c]	+++[h]
B(k)A	+[g]	+/-[g]	-[c]	
PY	-[a]	+[a]	-[a]	-[a]
CPP	++[i]	+[j]	+[j]	+[k]
B(a)P	+[g]	+[g]	++[c,l]	+++[h,m]

[a]IARC, 1983. [b]Kohan et al., 1985. [c]Mohapatra et al., in preparation. [d]Sanqaiah et al., in press. [e]Nesnow and Heidelberger, 1976. [f]Slaqa et al., 1978. [g]Nesnow et al., 1984b. [h]Nesnow et al., 1984a. [i]Eisenstadt and Gold, 1978. [j]Gold et al., 1980. [k]Cavalieri et al., 1981. [l]Nesnow et al., 1981. [m]Nesnow et al., 1983.

These divergent results in the various genetic toxicology bioassays have prompted us to study the metabolic activation of CP-PAH in these bioassay systems in an effort to probe their mechanisms of action.

METABOLISM STUDIES

Rat Liver Microsomes

The major microsomal metabolites of seven CP-PAH have been identified by UV, NMR, and mass spectrometric analyses and in some cases by comparison with authentic synthesized standards. For CPP, AA, AP, and B(j)A the major metabolite was identified as the dihydrodiol arising from oxidation and hydration at the cyclopenta-ring. This dihydrodiol was also present in microsomal incubates of B(l)A, B(e)A, and B(k)A. Although oxidation at the cyclopenta-fused ring is a major pathway of metabolism in CP-PAH it is not the only pathway. K-Region dihydrodiols of B(l)A, B(j)A, and CPP and the distal bay region dihydrodiol of B(j)A have been identified.

At present, two cyclopenta-ring oxides have been shown to exhibit genotoxic activity: CPP-3,4-oxide and B(l)A-1,2-oxide. Both oxides mutate S. typhimurium strain TA98 and both are active as gene mutagens in mammalian cells (Eisenstadt and Gold, 1978, Gold et al., 1980, Nesnow et al., 1984b). B(l)A-1,2-oxide has been estimated to account for up to 40% of the bacterial or mammalian cell mutagenic activity of B(l)A while CPP-3,4-oxide is estimated to be the only major contributor to the genotoxic activity of CPP.

C3H10T1/2CL8 Cells

Metabolism of [^{14}C]B(l)A and [^{3}H]B(j)A in C3H10T1/2CL8 cells has been studied (Mohapatra et al., in preparation). After 24 h of incubation the treated cells were lysed and the lysate and media extracted with organic solvents to remove the unconjugated metabolites. The aqueous layers were split and subjected to enzymatic hydrolysis by β-glucuronidase and aryl sulfatase. These incubates were then extracted with organic solvents. The solvent extracts were chromatographed by reverse-phase HPLC and the metabolites separated and quantitated. The metabolite rates of the major dihydrodiols as presented in Table 4 are the total of the unconjugated and conjugated forms.

TABLE 4. Metabolism of Benz(j)aceanthrylene and Benz(l)aceanthrylene by C3H10T1/2CL8 Cells[a]

B(j)A Dihydrodiol[b]				
1,2-Diol	9,10-Diol	11,12-Diol	Unk	Total
31	119	10	56	216

B(l)A Dihydrodiol[b]				
1,2-Diol	4,5-Diol	7,8-Diol	Unk	Total
33	36	280	273	622

[a]Cells were incubated with 10 µg/ml of the CP-PAH for 24 h.
[b]pmol total metabolite produced/h/10^6 cells.

B(j)A is metabolized to one major diol (B(j)A-9,10-diol) the diol distal to the bay-region, which represented 50% of the isolated metabolites. The cyclopenta-ring diol was formed to a small extent. The metabolism data suggests that in C3H10T1/2CL8 cells, activation to the bay-region diol-epoxide is a strong possibility for the activation of B(j)A. This also might explain the similar abilities of B(j)A and B(a)P to transform these cells, since the major B(a)P-DNA adduct in C3H10T1/2CL8 cells is the anti-B(a)P diol-epoxide guanine adduct (Brown et al., 1979).

B(l)A is metabolized at a rate 3 times that of B(j)A and the major metabolite formed is the K-region dihydrodiol, B(l)A-7,8-diol. K-Region metabolism is normally a detoxification route, since K-region arene oxides usually have low genotoxic activities compared to diol-epoxides. Oxidation at the cyclopenta-ring of B(l)A is a minor route in C3H-10T1/2CL8 cells. The decreased ability of B(l)A to transform C3H10T1/2CL8 cells compared to B(a)P which does not undergo K-region oxidation by C3H10T1/2CL8 cells (Nesnow et al., 1981) is, therefore, in concert with the metabolism results.

CONCLUSION

From metabolism studies with induced rat liver S9, a major pathway of metabolic activation seems to be via epoxidation of the cyclopenta-ring. Studies with the C3H10T1/2CL8 cell transformation assay suggest that alternate pathways may be operative. These studies need to be extended to identify the major DNA adducts formed in both systems to identify and confirm the mechanisms of activation of these CP-PAH.

Of the CP-PAH examined, all but one were more active than the parent PAH they were derived from (Table 3). CPP is more active than PY in bacterial mutation, morphological transformation, and mouse skin tumor initiation bioassays. Similarly, AA and AP are more active than A or P as bacterial mutagens and B(e)A, B(j)A, and B(l)A more active than BA in all bioassays examined. The increased activity of these CP-PAH and their identification in the environment prompt us to further study these mutagenic and potentially carcinogenic CP-PAH.

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